

Comparative evaluation of particle agglutination test for antibody to human immunodeficiency virus

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SUMMARY A comparative evaluation of a new serological test for human immunodeficiency virus (HIV) was carried out. The test used the agglutination of gelatin particles coated with HIV antigen. It was found to be more sensitive than current enzyme linked immunosorbent assays (ELISAs) and more specific than the western blot test. Because of its simplicity, it promises to be of value, especially in developing countries.

Enzyme linked immunosorbent assays (ELISAs) for antibody to human immunodeficiency virus (HIV) are widely used to diagnose and control HIV infection. Many of these tests have high levels of sensitivity and specificity.¹ Although these tests are useful, they are technically difficult to perform and require expensive equipment, such as a spectrophotometer and a plate washer.

A new serological test that overcomes many of these problems has been described.² It is an agglutination assay in which tanned gelatin particles have been coated with HIV antigen. The test involves a one step reaction of antibody to antigen. The particles are dyed so that the results can be read visually.

After a preliminary evaluation had shown that the test was promising, we carried out a more extensive study. This paper presents our findings.

Materials and methods

The particle agglutination test was supplied by Fujirebio Inc, Tokyo. The kit came with a microtitre plate, two reagent droppers, and the necessary reagents. Basically, the test consisted of preparing serum dilutions in the microtitre plate, and adding sensitised and unsensitised particles to the appropriate serum dilutions. After being incubated for two hours, the plate was read macroscopically for agglutination.

The test results were compared with those of the Abbott enzyme immunoassay (EIA) and Abbott recombinant EIA (REIA) and ELISAs of the following companies: Behring, Diagnostic Biotechnology,

Electro-Nucleonics, Pasteur, Roche, Sorin (plate), and Wellcome. The antigens for the Abbott REIA and Roche ELISA were prepared by DNA recombinant technology.

The confirmatory tests used were the Diagnostic Biotechnology western blot, Commonwealth Serum Laboratories western blot, and the Abbott confirmatory EIA (CEIA). The Abbott CEIA uses a competitive immunoassay method in which polystyrene beads are separately coated with p24 and gp41 recombinant antigens. We tested for HIV antigen by a solid phase sandwich type enzyme immunoassay for p24 which was produced by Abbott Laboratories.

The methods adopted for all the tests were according to the manufacturers' instructions. As not all the tests were available throughout the study period, the particle agglutination test was compared with those that were available at a particular time.

The serum samples came from different sources. One batch of 400 was from routine samples sent for HIV screening during a three week period. These were from prostitutes, high risk groups, and other patients. Another 103 serum samples were collected during an 18 month period. They had shown either initial or repeated reactivity in the ELISA and had been kept at -20°C for confirmatory tests. Ten serum samples that gave positive results for antinuclear antibodies were also tested. We used two familiarisation serum panels (from Abbott Laboratories and Electro-Nucleonics).

Results

The reactivity patterns of the particle agglutination test were similar to those of the haemagglutination tests. For almost all the serum samples tested there was no difficulty in differentiating positive from negative results. Only two samples gave indeterminate results.

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Table 1 Evaluation of human immunodeficiency virus (HIV) antibody test kits with two HIV antibody positive serum samples (numbers are serum titres)

HIV tests	HIV antibody positive serum samples	
	D845	HT558
Abbott EIA	1/256	1/4096
Abbott REIA	Not tested	1/8192
Behring	1/256	Not tested
Diagnostic Biotechnology ELISA	Not tested	1/4096
Electro-Nucleonics ELISA	1/512	1/1024
Particle agglutination	1/131072	1/32768
Pasteur ELISA	1/1024	Not tested
Roche ELISA	Not tested	1/1024
Sorin ELISA	Not tested	1/1024
Wellcome ELISA	1/512	1/4096
Diagnostic Biotechnology western blot:		
p24 antibody	Not tested	1/4096
gp41 antibody	Not tested	1/256
Commonwealth Serum Laboratories western blot:		
p24 antibody	Not tested	1/8192
gp41 antibody	Not tested	1/128
Abbott CEIA:		
Core	Not tested	1/2048
Env	Not tested	1/4096

EIA = enzyme immunoassay.

REIA = recombinant EIA.

ELISA = enzyme linked immunosorbent assay.

CEIA = confirmatory EIA.

On repeating the test, both gave negative results.

Table 1 shows the reactivity of various HIV tests on twofold dilutions of two strongly reactive serum samples, D845 and HT558, prepared in normal human serum. The various ELISA kits had different levels of sensitivity. The differences between the most and least sensitive kits were fourfold for D845 and eightfold for HT558. The particle agglutination test was four and 128 times more sensitive than the most sensitive ELISA kits for the two serum samples.

The results also showed that the western blot kits were more sensitive for p24 antibody, and much less sensitive for gp41 antibody, than the Abbott CEIA.

Table 2 shows the reactivity of various ELISA kits and the particle agglutination test on two sets of serum panels supplied by commercial firms. The particle agglutination and the two Abbott tests gave 100%

correlation with the results as assessed by the panel suppliers.

Table 3 shows the reactivity of the Diagnostic Biotechnology western blot, Abbott CEIA, and particle agglutination tests on 103 problem serum samples, which had given initial or repeated reactivity with the ELISAs and had been sent for confirmation. Fifteen could be classified as positive, as the western blot test showed bands at p24 and gp41. Thirteen of them also had a band at gp120. They all gave repeated reactivity with the ELISA. All were positive by the Abbott CEIA and the particle agglutination test. HIV antigen was detected in three samples.

The western blot results for 23 serum samples were indeterminate, as the *env* bands were absent. Twenty of the serum samples were repeatedly reactive with the ELISA, and three showed only initial reactivity. All 23

Table 2 Evaluation of human immunodeficiency virus (HIV) antibody test kits with two sets of serum panels

HIV tests	Abbott panel		Electro-Nucleonics panel	
	Positive (n = 10)	Negative (n = 9)	Positive (n = 4)	Negative (n = 8)
Abbott EIA	10 positive	9 negative	Not tested	Not tested
Abbott REIA	Not tested	Not tested	4 positive	8 negative
Behring ELISA	5 pos, 5 neg	9 negative	Not tested	Not tested
Electro-Nucleonics ELISA	7 pos, 3 neg	9 negative	4 positive	8 negative
Particle agglutination	10 positive	9 negative	4 positive	8 negative
Pasteur ELISA	9 pos, 1 neg	9 negative	Not tested	Not tested
Roche ELISA	Not tested	Not tested	2 pos, 2 neg	8 negative

EIA = enzyme immunoassay.

REIA = recombinant EIA.

ELISA = enzyme linked immunosorbent assay.

Table 3 Reactivity of three human immunodeficiency virus (HIV) antibody tests on 103 problem serum samples

Diagnostic Biotechnology western blot	No	Positive results in the:	
		Abbott confirmatory enzyme immunoassay	Particle agglutination test
Positive (bands at p24 and gp41 (and gp120 in 13))	15	15*	15
Indeterminate (bands at p15, p24, p55, and p64)	23	0	1
Negative (no visible bands)	65	0	0

*All were *core* and *env* antibodies positive except five, which were *env* positive but *core* negative.

were negative with the Abbott CEIA, and 22 were negative by the particle agglutination test. The single particle agglutination positive serum sample was repeatedly reactive with the ELISA, and showed bands at p15 and p55 in the western blot test. All the indeterminate serum samples were negative for HIV antigen.

Two serum samples that were Abbott CEIA and particle agglutination negative, and had p24 bands only, were serially diluted in normal human serum. The p24 bands were still detectable at a titre of 1/16 in the western blot test.

No band was detected for 65 sera in the western blot, and all were negative with the Abbott CEIA and particle agglutination tests. Only 29 of them were repeatedly reactive with the ELISA.

Particle agglutination was tested in parallel with the Abbott REIA on 400 routine specimens. These all gave negative results by particle agglutination, but one serum sample was positive by the Abbott REIA. This serum sample did not produce any band in the western blot test and was also negative by the Abbott CEIA.

Fifty of the negative serum samples were heat treated at 56°C for 30 minutes and tested again by particle agglutination. They all remained negative.

Ten serum samples that were positive for antinuclear antibodies were also evaluated. Immunofluorescence had shown five with homogeneous, three with speckled, one with peripheral, and one with centromere patterns. They were all negative with particle agglutination and the Abbott REIA. One was positive with the Electro-Nucleonics ELISA.

Discussion

The present study shows that the particle agglutination test is very sensitive in detecting antibodies to HIV. It detected antibodies in two positive serum samples at much higher titres than the current ELISA systems. It also correctly identified all the positive serum samples from the two serum panels supplied by commercial firms. All 15 samples that were repeatedly reactive by the ELISA and showed p24 and gp41/120 bands in the western blot, gave positive results with

particle agglutination. These serum samples also yielded positive results with the Abbott CEIA.

More sensitive tests are needed to close the diagnostic "window" between the dates of infection and antibody detection.³ It is at present premature to think that particle agglutination will fill this gap. Sequential studies on serum taken from patients who have been exposed recently to HIV will need to be carried out to assess whether the test will detect antibodies sooner than the ELISA.

The current practice in many laboratories is to verify ELISA positive serum with the western blot test. Of the 103 serum samples tested by the western blot, Abbott CEIA, and particle agglutination, the latter two tests correctly identified all the serum samples that gave unequivocally positive or negative results by the western blot test.

Of 23 serum samples that gave indeterminate western blot results, all were negative with the Abbott CEIA, and one was particle agglutination positive. None was from high risk groups or people with symptoms of HIV infection. All samples were negative for HIV antigen. These samples gave p15, p24, or p55 bands, but no bands at gp41 or gp120. False positive western blot results at such band sites have been reported previously.^{4,6} In a study of blood donors, six were followed up for about two years, and no HIV infection was found.⁵ Recipients of the blood from these donors remained well. Culture of the donors' cells for HIV gave negative results.

The nature of the false positive western blot results has been attributed to cross reactions with normal cell constituents or other human putative retroviruses.⁴ The Abbott CEIA uses p24 and gp41 antigens that have been produced by recombinant DNA technology. Because of this, there is little danger of cross reactivity with normal cellular components. Specificity should therefore be higher than that of the western blot.

Our results show that the western blot test is better able to detect p24 than gp41 antibodies. It is therefore possible for a serum with low antibody titres to yield a p24 band but no gp41 band. As the western blot test is more sensitive than the Abbott CEIA in detecting p24

antibody, the latter assay might even be negative. In our study of titrations of two indeterminate serum samples, however, western blot p24 bands were seen even at titres of 1/16. As the Abbott CEIA detected p24 antibody at only one or two serum dilutions lower, the test should have given positive results had there been HIV p24 antibody present. Thus the Abbott CEIA is useful in analysing results that are indeterminate on western blot. The specificity of particle agglutination is higher than that of the western blot, and approaches that of the Abbott CEIA.

The high specificity of particle agglutination is shown further by the negative results with 10 serum samples that had antinuclear antibodies, and 400 samples from prostitutes and patients. One of the autoimmune samples was positive by the Electro-Nucleonics ELISA, and one of the 400 samples was positive by the Abbott REIA. Both were negative on western blotting. Heat inactivation of 50 serum samples did not affect the outcome of particle agglutination.

The present study shows that the particle agglutination test has high levels of sensitivity and specificity. It is simple to perform and does not require sophisticated

equipment. It is particularly suitable for laboratories in developing countries.

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